Glutathione S-Transferase μ Genotype, Diet, and Smoking as Determinants of Sister Chromatid Exchange Frequency in Lymphocytes[1]

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Abstract

Polymorphisms in inherited metabolic traits and intake of dietary antioxidants have been reported to be associated with risk for the development of lung cancer in smokers. This increased risk of lung cancer is presumably attributable to the accumulation of DNA damage. We conducted a study to investigate whether genetic metabolic variants and antioxidant consumption affected the sister chromatid exchange (SCE) level in lymphocytes. Study subjects were 78 friends and spouses of cases from a case-control study of lung cancer designed to investigate the association of metabolic polymorphisms with lung cancer. The metabolic traits studied included glutathione S-transferase class μ and variants of P-450 isoenzymes CYP1A1 and CYP2D6. Intake of antioxidants including vitamins A, C, and E and selenium was determined through the administration of a validated, semiquantitative food frequency questionnaire. Detailed information on smoking, family history of cancer, medical history, and environmental and occupational exposures was also obtained in an interviewer-administered questionnaire. Smoking status was found to be significantly associated with SCE frequency. In addition, SCE frequency decreased with the period of time since quitting smoking. The presence of one or more glutathione S-transferase class μ alleles was associated with significantly lower SCE. Higher intake of vitamin A and selenium was also inversely associated with SCE level. Thus, the results suggest that glutathione S-transferase class μ and the intake of vitamin A and selenium may modulate the accumulation of chromosomal damage in lymphocytes.

Introduction

Cigarette smoking accounts for 30% of all cancer deaths in the United States; most of these deaths are from lung cancer (1). The current estimate of 5-year survival of non-small cell lung cancer is only 10–15% (2); thus, it is important to prevent lung cancer by reducing exposure and possibly intervening in the carcinogenic process at an early stage. The fact that most smokers do not develop lung cancer suggests that there may be endogenous and exogenous differences in individuals that are important in protecting smokers from the development of cancer. Many studies have suggested an important role for inherited metabolic genes (3–5) and antioxidants in the genesis of smoking-induced lung cancer (6–8).

Cigarette smoke contains more than 40 known or putative carcinogens (1). PAHs,[3] which are abundant in cigarette smoke, require Phase I metabolic activation to generate DNA damage in smokers (9). These active metabolites then become a substrate for Phase II conjugation, resulting in detoxification of reactive metabolites (9). Thus, individuals with inherited variant metabolic enzyme activities might have an altered risk of cigarette smoke-induced cancers (10).

Several genes have been found to be important in the activation of procarcinogens in cigarette smoke, including cytochrome P-450 isoenzymes CYP1A1 and CYP2D6 (4, 11). The polymorphisms in the *CYP1A1* gene have been associated with the risk of lung cancer in the Japanese, but not in Caucasians (12–14). Polymorphisms in another P-450 gene, *CYP2D6*, has also been associated with the occurrence of smoking-induced lung cancer (15–17). GST-μ is a Phase II metabolic enzyme responsible for detoxifying electrophiles through the conjugation of reactive metabolites with glutathione. Approximately 50% of Caucasians have a homozygous deletion of this gene and, therefore, do not have this enzyme. Epoxides of some PAHs have been found to be substrates for GST-μ (18). Many studies have reported an association of the GST-μ-null genotype with the risk of lung cancer and other smoking-related cancers, including bladder and laryngeal cancers in smokers. (12, 19–21). Some workers, however, have not found a similar association between GST-μ and lung cancer (22, 23).

Although the association of polymorphisms in metabolic genes with the occurrence of lung cancer has been widely studied, little data are available that address the association of the variant metabolic genes with genetic damage. Previous work has demonstrated that SCE induction by *trans*-stilbene oxide was higher in lymphocytes deficient in GST-μ activity (24). In addition, an association between the GST-μ-null genotype and an elevated PAH adduct level was reported recently

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; SCE, sister chromatid exchange; GST-μ, glutathione S-transferase class μ; HFC, high frequency cell; dNTP, deoxynucleotide triphosphate.

in a study of 30 subjects (25). van Poppel *et al.* (26) also found that heavy smokers without GST-μ activity had higher SCE frequencies in lymphocytes than those who had GST-μ activity.

Diet is an additional important source of exogenous factors that may affect the risk of lung cancer. Most notably, many studies have found an inverse association of vegetable and fruit intake and the risk of lung cancer (27–29). Vitamins A, C, and E, and carotenoids in fruit and vegetables have been of interest because of their potential role in cancer prevention, particularly in the prevention of lung cancer (6, 7). Selenium as an antioxidant has been shown to be inversely associated with the risk of lung cancer (8). Pryor (30) has suggested that free radicals generated by cigarette smoking may be initiators and promoters in lung carcinogenesis. Antioxidants may exert their protective effect by "neutralizing" these free radicals in cigarette smoke (31).

The effect of antioxidant consumption on SCE also has been studied previously. Vitamin C administration suppressed SCE induction by oxygen radicals in Chinese hamster ovary cells (32). β-Carotene also has been reported to decrease SCE induced by several chemicals in mouse mammary cells (33). However, a double-blind trial did not show reduced SCE in smokers after 14 weeks of administration of β-carotene (34). Another study in smokers also did not demonstrate the reduction in SCE frequency by β-carotene (35). At the same time, administration of β-carotene was reported to be associated with reduced micronucleus frequency in sputum from cigarette smokers (36).

In an effort to examine the genetic effect of these exogenous and endogenous factors, we chose to study a chromosomal end point as a marker of induced genotoxic damage. Chromosomal damage is believed to represent a crucial stage of carcinogenesis. SCE has been demonstrated in many studies to be a useful tool to detect the DNA damage from carcinogen exposure (37, 38).

In this report, we studied SCE frequency in lymphocytes to assess the effect of polymorphisms in metabolic traits and dietary antioxidant consumption.

Materials and Methods

Study Population

This study has been approved by the Human Subjects Committee at both the Massachusetts General Hospital and the Harvard School of Public Health. The study population was control subjects enrolled in a case-control study of lung cancer designed to investigate whether polymorphic metabolic traits are associated with lung cancer. The controls were friends or spouses of lung cancer cases, and some were friends or spouses of cardiovascular patients admitted to the same hospital. Lung cancer patients were newly diagnosed with operable primary lung cancer. From December 1992 to April 1994, 141 controls had been enrolled. Slides for SCE were made for all subjects. Interviewer-administered questionnaires were also given to each control. Because of the high prevalence of former smokers in the population, we analyzed data from all of the current smokers, all of the controls who had never smoked, and a randomly selected group of former smokers. We studied a total of 81 subjects. Due to technical problems and inadequate questionnaire information, three subjects were excluded. Overall, 78 controls were available for analysis. We chose to analyze this number of controls because this gave us a >80% chance to detect a 0.5 SCE/cell difference between GST-μ-null and GST-μ-positive genotypes. These subjects were generally healthy, but 48 were taking medications including β-blockers, calcium channel blockers, and nonsteroid antiinflammatory agents.

SCE Assay

Blood was collected in heparinized tubes, transferred to our laboratory the same day, and processed within 24 h. One ml of whole blood was added to 9 ml of RPMI 1640 culture medium with L-glutamine containing 10% fetal bovine serum (Hyclone), 1% phytohemagglutinin-P (Burroughs-Wellcome), 100 units/ml penicillin, and 100 μg/ml streptomycin. Fifty μM 5-bromodeoxyuridine was added to the culture at 24 h to achieve differential staining. The culture was maintained at 37.5°C in 5% CO_2 for 72 h. Colcemid (0.1 μg/ml) was added 1.5 h before harvesting. The lymphocytes were harvested with hypotonic KCl solution (75 mM) for 10 min. Cells were then fixed in methanol:acetic acid (3:1, v/v). The slides were prepared by the air-dry technique. The chromosomes were stained by a modification of the fluorescence-plus Giemsa technique of Perry and Wolff (39). Each slide was stained in Hoechst 33258 (5 μg/ml) for 10 min. Then slides were mounted with Sorensen's solution (pH 6.8) and irradiated with the black light from two 15-W tubes for 10 min and stained with 5% Giemsa for 5 min. For each subject, 50 metaphases were scored to determine mean SCE frequency. Mean SCE for HFC was calculated from the highest five metaphases for each study subject. All slides were scored by one reader blind to the status of the subjects.

Genotyping of Polymorphic Metabolic Traits

GST-μ. GST-μ genotype was determined by a modification of the method of Comstock *et al.* (40). Primers hybridizing to the 5′ region of exon 4 (5′-CTGCCCTACTTGATTGATGGG-3′) and the 3′ region of exon 5 (5′-CTGGATTGTAGCAGATCATGC-3′) of GST-μ were used to amplify a 273-bp fragment. The reaction solution included 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 1% Triton X-100, 0.2 mM dNTPs, 200 ng of primers, 1 μl of DNA template (whole blood), and 2.5 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). PCR conditions were 95°C for 4 min, followed by 30 cycles of 94°C for 1 min, 61°C for 30 s, and 72°C for 1 min. The reaction product was electrophoresed in 2% agarose. Individuals with one or more GST-μ alleles had a 273-bp product, whereas individuals with the GST-μ-null genotype had no fragment. Control primers that amplify human β-actin (493 bp) were also included in each reaction to confirm the presence of amplifiable DNA in the samples. β-actin primers were 5′-GGGCACGAAGGCTCATCATT-3′ and 5′-GGCCCCTCCATCGTCCACCG-3′.

CYP1A1-*Msp*I Polymorphism. A modified method from Kawajiri *et al.* (41) was used to identify CYP1A1 genotypes. Primers hybridizing to the 5′ region included bases 42–65 (5′-TCACTCGTCTAAATACTCACCCTG-3′), beginning from the poly(A) signal, whereas the 3′ primers were from bases 435–455 (5′-TAGGAGTCTTGTCTCATGCCT-3′). Primers used for reamplification "nesting" were bases 116–136 (5′-CAGTGAAGAGGTGTAGCCGCT-3′) and bases 387–410 (5′-GAGGCAGGTGGATCACTTGAGCTC-3′), again counting from the poly(A) signal. With the use of these primers, fragments of 135 and 160 bp were generated after amplification and digestion with *Msp*I endonuclease when mutations were present. DNA was obtained with the use of Chelex solution as described by Walsh *et al.* (42). Ten μl of Chelex DNA were added to a PCR buffer containing 150 ng of primers, 2.0 mM $MgCl_2$, 200 μM of dNTPs,

50 mM KCl, 20 mM Tris-HCl (pH 8.6), and 0.1% BSA in a final volume of 50 μl. Reaction mixtures were preincubated for 4 min at 95°C. Two and one-half units of Taq polymerase (Perkin Elmer Cetus) were then added at 80°C. PCR conditions were 94°C for 25 s and 60°C for 25 s, followed by 70°C for 25 s. Thirty-five rounds of amplification were performed before digestion with *Msp*I was complete for 3–4 h at 37°C. The products were then electrophoresed on 2.5% agarose gel to allow unambiguous detection with ethidium bromide staining. Homozygous wild-type individuals had a PCR product of 295 bp, whereas homozygous-rare allele individuals lacked the large 295-bp band and had the smaller bands (160 and 135 bp), and heterozygous individuals had three bands (295, 160, and 135 bp).

CYP1A1-Isoleucine/Valine Variant. The wild-type sequence immediately adjacent to the mutation is ACC ATT GCC CGC TGG GAG GTC TTT. The mutation is the replacement of an A by a G, resulting in the isoleucine to valine change. The genotyping used nested primers, amplifying a portion of exon 7 around the point mutation. For the second round of amplification, a mismatch primer at the penultimate base pair was used to create a *Sty*I excision site, and thus, generating an informative RFLP. The outer primers were 5′-GAACTGCCACTTCAGCTGTCT-3′ and 5′-CAGCTGCATTTGGAAGTGCTC-3′. Ten μl of Chelex DNA were added to a PCR buffer containing 150 ng of primers, 20 mM Tris-HCl (pH 8.6), 50 mM KCl, 2.5 mM $MgCl_2$, 1% BSA, and 200 μM of dNTPs. Reaction mixtures were preincubated for 4 min at 95°C, then 2.5 units of Taq polymerase were added at 80°C, followed by 40 rounds of PCR amplification (94°C for 25 s, 60°C for 25 s, and 70°C for 25 s). This amplification resulted in a 312-bp product that was subject to a second round of amplification. The upstream primer for the second round was 5′-CTATCGACAAGGTGTTAAGT-3′. The second primer was designed to create an RFLP with the use of the following primer where the penultimate base at the 3′ end was a mismatch by design (a C instead of an A): 3′-ACCGGGCGACCCTCCAGAAA-5′. This amplification was carried out under the conditions noted above, except that the annealing temperature was 66°C. This resulted in a 92-bp product when the mutation was not present that contained the restriction site for *Sty*I endonuclease. When the mutation was present, the fragment was 114 bp.

CYP2D6-G to A Variant. Mutant *CYP2D6* alleles were identified by a modified PCR assay by Gough *et al.* (43). Primers were 5′-GCCTTCGCCAACCACTCCG-3′ and 5′-AAATCCTGCTCTTCCGAGGC-3′. Ten μl of Chelex DNA were added to a PCR buffer containing 150 ng of primers, 1.25 mM $MgCl_2$, 0.2 mM of dNTPs, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), and 0.1% BSA in a final volume of 50 μl. Reaction mixtures were preincubated for 4 min at 95°C, and then 2.5 units of Taq polymerase (Perkin Elmer Cetus) were added at 80°C. Subsequently, 40 rounds of amplification were performed at 94°C for 30 s, 63°C for 30 s, and then at 72°C for 1 min. The PCR products were digested with *Bst*NI for 2 h at 60°C and then electrophoresed on 2.5% agarose gel. Homozygous-rare allele individuals had product fragments of 335 bp, whereas homozygous wild-type individuals had 230- and 105-bp fragments, and heterozygous individuals had all three fragments.

Smoking History

Smoking history was obtained with the use of a modified version of the standard respiratory questionnaire of the American Thoracic Society (New York; 44). The smoking history included cigarettes smoked daily for current smokers, pack-years of cigarettes smoked, the period of years since quitting smoking, and the duration of smoking. Pack-years, an indicator of cumulative smoking dose, was defined as cigarette packs smoked daily multiplied by the years of smoking. Current smokers were defined as smokers who had been smoking through 1 month before blood collection.

Dietary History

Intake of antioxidants, including vitamins A, C, and E, as well as selenium, folate, alcohol, caffeine, and other nutrient components, was obtained from a Harvard Food Frequency Questionnaire developed by Willett *et al.* (45). This semiquantitative food frequency questionnaire was composed of 121 food and beverage items with the additional use of vitamin and mineral supplements. The amount of nutrient intake is calculated by multiplying the frequency of the specific unit of food by the contents of nutrients in that unit of food. Vitamin A intake was the sum of the consumption of carotenoids and retinol.

Occupational, Environmental, and Medical History

Occupational and environmental exposures were assessed by questionnaires. Asbestos exposure was quantified according to the year of exposure, duration of exposure, and the nature of job activity. A family history of cancer within first-degree and other relatives and medical history, including medications, were also obtained.

Statistical Analysis

Crude comparisons of SCE frequency by variables of interest, including smoking status, age, sex, dietary intake (folate, vitamins A, C, and E, as well as caffeine and alcohol), and polymorphisms of metabolic traits (GST-μ, CYP1A1, and CYP2D6), as well as occupational and environmental exposure to asbestos and family history of cancer were conducted with the use of Student's *t* test. Subsequent analysis used an ANOVA to fit a model with the dependent variable (SCE) as a function of smoking, age, sex, dietary factors, and metabolic traits. For CYP1A1 and CYP2D6, homozygous and heterozygous variant carriers were pooled for analysis.

Results

There were 78 subjects included in the analysis. Demographic characteristics of study subjects are summarized in Table 1. Of the study population, current smokers accounted for 23%, former smokers, 49%, and never smokers, 28%. The mean age of subjects was 58 ± 12 (SD) years. Females accounted for 60% of subjects. Most subjects were Caucasians (95%). Thirty-five subjects (44%) had one or more GST-μ functional alleles. Eight subjects (10%) had 1 or more variant alleles at the *Msp*I site in CYP1A1, and 16 subjects (20%) had 1 or more variant alleles in CYP1A1, resulting in 1 amino acid change from isoleucine to valine. Homozygous and heterozygous G to A mutations in CYP2D6 were found in 31 subjects (40%). The overall mean SCE frequency was 7.5 ± 1.1 (SD). SCE frequency by smoking history, dietary intake of antioxidants, and metabolic genotypes is summarized in Table 2. Crude comparisons of SCE frequency with the use of Student's *t* test were performed on variables of interest. Females had higher SCE than males (7.6 *versus* 7.2), although this was not statistically significant. SCE frequency was highest in age groups of 50–65 years, and lowest in the group of >65 years; however, this difference did not reach significance. Current smokers had 8.4

Table 1 Demographic characteristics of the study population

	$n = 78$
Age (yr) (± SD)	58 ± 12
Gender (female, %)	60
Caucasians (%)	95
Smoking	
Current smokers (%)	23
Cigarettes/day	19 ± 13
Pack-years	37 ± 21
Yrs since smoking	37 ± 8
Former smokers (%)	49
Pack-years	32 ± 37
Years since smoking	42 ± 11
Years since quitting	19 ± 14
Never smokers (%)	28
GST-μ, null type (%)	56
CYP1A1 (%)	
*Msp*I[a]	10
Iso/Val[b]	20
CYP2D6[c] (%)	40
Caffeine (mg/day)	321 ± 253
Alcohol (g/day)	11 ± 21
Vitamin A (IU/day)	15100 ± 9700
Vitamin C (mg/day)	367 ± 450
Vitamin E (mg/day)	115 ± 224
Carotenoid (IU/day)	11100 ± 7350
Retinol (IU/day)	3990 ± 5050
Selenium (μg/day)	4 ± 23
Folate (μg/day)	454 ± 268
Asbestos exposed (%)	11
Family history of cancer (%)	50

[a] One or more mutations at *Msp*I in CYP1A1.
[b] One or more mutations (isoleucine to valine) in CYP1A1.
[c] One or more G to A mutations in CYP2D6.

SCE/cell, former smokers, 7.4 SCE/cell, and never smokers, 6.9 SCE/cell. SCE frequency was higher in subjects with higher pack-years by smoking status, but this also did not reach a statistical significance. When SCE frequency was compared with the use of Student's *t* test by GST-μ genotypes in current, former, and never smokers, subjects with the GST-μ-null genotype had higher SCE frequency, which did not reach statistical significance. In addition, SCE frequency was lower in subjects with high intake of folate, selenium, vitamins A, C, and E, as well as carotenoids and retinol. This difference of SCE frequency among dietary components was more prominent in current smokers, although it was not significant in the crude comparison. Alcohol and caffeine consumption were not significantly associated with SCE. A family history of cancer was not associated with SCE when family history of first-degree relatives or all known relatives was used for the comparison of SCE. The exposure to asbestos and other occupational and environmental mutagens or carcinogens was not associated with an increase in SCE frequency. Similarly, analysis revealed no association of the medications used by the subjects with SCE frequency. In addition, 33 subjects had history of dental X-ray exposure; however, this was not associated with SCE frequency.

An ANOVA was next used to investigate the factors associated with SCE frequency. This was fit stepwise with SCE frequency as the dependent variable, with the use of age, gender, smoking, dietary components, polymorphic metabolic traits, environmental and occupational exposures, and medical and family history of cancer as candidate predictor variables. Age, gender, pack-years, years-quit, GST-μ, and vitamin A fit the model best for SCE frequency (Table 3). In this analysis, age was modeled with the use of two terms because the frequency plot suggested a plateau in an age-associated increase in SCE at age 65 years. This model explained 40% of the variance in SCE frequency.

In the data set, including current and never smokers, SCE frequency was significantly associated with cigarettes smoked daily ($P < 0.01$; ANOVA) after adjusting for age, gender, vitamin A, and GST-μ. When pack-years replaced cigarettes smoked daily in the model, SCE frequency remained significantly associated with pack-years ($P < 0.01$; ANOVA; Fig. 1). In analysis confined to current and former smokers, the number of years since quitting was inversely associated with SCE frequency ($P = 0.06$; ANOVA; Fig. 2) after adjusting for age, gender, pack-years, and GST-μ, as well as vitamin A. Overall, smoking (pack-years and the number of years since quitting) accounted for 25% of variance on SCE frequency in all study subjects (Table 3). The group of subjects ages 50–65 had significantly higher SCE frequency than the group of subjects ages >65 years ($P < 0.05$; ANOVA) after adjusting for gender, smoking, vitamin A, and GST-μ; the group of subjects <50 years of age had higher SCE frequencies than the group of subjects ages 65 years and older, although this was not significant. SCE frequency was significantly higher in females ($P = 0.01$; ANOVA). Overall, there was no seasonal change of SCE after controlling for gender, smoking, GST-μ, and vitamin A intake.

The association of GST-μ genotype and SCE frequency was significant after adjusting for age, gender, smoking, and vitamin A intake ($P = 0.05$; ANOVA). Overall, the slopes of linear regression comparing smoking "dose" (pack-years and years since quitting) and SCE between GST-μ genotypes were not significantly different. There was no association between SCE frequency and polymorphisms in CYP1A1 (*Msp*I or *Iso/Val*) or CYP2D6.

Intake of vitamin A was significantly associated with SCE frequency after adjusting for age, gender, smoking, and GST-μ ($P = 0.05$; ANOVA). When selenium replaced vitamin A in the model, SCE frequency was also significantly higher in the group with higher intake of selenium ($P = 0.05$; ANOVA) after adjusting for age, gender, smoking, and GST-μ. However, when conservative analysis was performed, by removing individuals who supplemented their diet with antioxidants, neither vitamin A nor selenium was significantly associated with SCE frequency. Intake of other antioxidants (vitamins C and E) and folate were not significantly associated with SCE frequency in the model. Alcohol and caffeine consumption were also not associated with a significant increase in SCE frequency.

The mean SCE of the highest five metaphases was also calculated to derive a mean for HFCs. The association of HFC frequency with gender and smoking remained unchanged in the model. The final model is summarized in Table 4; this model explained 35% of the variance in the HFC frequency.

Table 2 SCE frequency in controls by smoking status, gender, metabolic trait, and diet

Variables	Never		Former		Current		All	
	n	SCE (SD)	*n*	SCE (SD)	*n*	SCE (SD)	*n*	SCE (SD)
All	22	6.9 (0.9)	38	7.4 (0.9)	18	8.4 (1.2)	78	7.5 (1.1)
Age (yr)								
<50	4	6.8 (0.6)	8	7.1 (0.9)	6	8.1 (1.2)	18	7.4 (1.1)
50–65	5	7.5 (0.8)	18	7.6 (1.0)	12	8.5 (1.3)	35	7.9 (1.2)
>65	13	6.6 (0.9)	12	7.2 (0.9)			25	6.9 (1.0)
Gender								
Female	15	7.2 (0.9)	22	7.4 (0.9)	10	8.7 (1.3)	47	7.6 (1.1)
Male	7	6.2 (0.5)	16	7.3 (1.1)	8	8.0 (1.1)	31	7.2 (1.1)
GST-μ[a]								
Null	8	7.3 (0.8)	24	7.4 (0.9)	10	8.4 (1.5)	42	7.6 (1.1)
Presence	14	6.6 (0.9)	14	7.2 (1.1)	7	8.3 (0.8)	35	7.2 (1.1)
Pack-years[b]								
0	22	6.9 (0.9)					22	6.9 (0.9)
<20			18	7.0 (0.7)	4	7.7 (1.0)	22	7.1 (0.7)
20–50			10	7.7 (0.9)	10	8.4 (1.3)	20	8.0 (1.0)
>50			9	7.9 (1.0)	4	9.2 (1.1)	13	8.3 (1.2)
Folate (μg/day)								
<300	6	6.8 (0.6)	13	7.6 (1.1)	6	9.2 (0.9)	25	7.8 (1.2)
>300	16	6.9 (1.0)	25	7.2 (0.9)	12	8.0 (1.2)	53	7.2 (1.0)
Carotenoids (IU/day)								
≥10,000	11	6.9 (0.9)	17	7.4 (1.0)	9	8.0 (1.3)	37	7.4 (1.1)
<10,000	11	6.9 (1.0)	21	7.4 (1.0)	9	8.8 (1.0)	41	7.5 (1.1)
Retinol (IU/day)								
≥4,000	9	7.3 (1.1)	13	7.3 (0.9)	5	8.0 (1.4)	27	7.4 (1.0)
<4,000	13	6.6 (0.7)	25	7.4 (1.0)	13	8.6 (1.2)	51	7.5 (1.2)
Vitamin A (IU/day)								
≥13,000	11	6.9 (1.1)	15	7.3 (1.0)	5	7.9 (1.2)	31	7.2 (1.0)
<13,000	11	6.9 (0.8)	23	7.4 (1.0)	13	8.6 (1.2)	47	7.6 (1.2)
Vitamin C (mg/day)								
≥350	6	7.1 (1.1)	9	7.6 (1.0)	4	8.1 (1.8)	19	7.5 (1.1)
<350	16	6.8 (0.8)	29	7.3 (1.0)	14	8.5 (1.1)	59	7.4 (1.1)
Vitamin E (mg/day)								
≥10	16	6.8 (1.0)	18	7.3 (1.0)	9	8.1 (1.2)	43	7.3 (1.1)
<10	6	7.0 (0.8)	20	7.4 (0.9)	9	8.7 (1.1)	35	7.6 (1.1)
Selenium (μg/day)								
≥4	2	6.9 (0.3)	4	7.3 (1.1)	2	7.6 (1.0)	8	7.3 (0.8)
<4	20	6.8 (0.9)	34	7.3 (0.9)	16	8.5 (1.2)	70	7.4 (1.1)

[a] GST-μ genotype can not be determined in one subject.
[b] One subject had no pack-yr information.

Table 3 Multiple regression model for SCE

	Regression coefficient	SE	*P*
Intercept	8.2	0.4	<0.01
Age1[a]	0.68	0.24	<0.01
Age2[b]	0.40	0.29	0.1
Gender (male)	−0.61	0.23	0.01
Pack-years	0.015	0.003	<0.01
Yrs-quit	−0.013	0.007	0.09
GST-μ-null genotype	0.43	0.21	0.05
Vitamin A (1000 IU/day)	−0.023	0.011	0.05

[a] Reference group, age ≥ = 65 years; age 1 = 50–65 years.
[b] Reference group, age ≥ 65 years; age 2 ≤ 50 years.

Discussion

In our study, we found that age, gender, smoking, and GST-μ status, as well as vitamin A and selenium intake, were associated with SCE frequency. Smoking habits were highly associated with SCE frequency; current smokers had higher SCEs than former and never smokers, and pack-years smoked were also associated with elevations in SCE frequency. This has been reported previously (46–49). Previous reports on the short-term effect of smoking cessation on SCE frequency have been inconsistent (50–52). The apparent linear relationship between SCE frequency and the number of years since quitting smoking in this study suggests that damage accumulates and then recedes over several decades. In this study, smoking accounted for approximately 25% of variance, consistent with the results of other investigators (48, 53).

In the current study, SCE frequency in females was approximately 0.5 SCE/cell higher than that in males, also consistent with previous studies (48, 54). The mechanism responsible for this is unclear. Females have an extra X chromosome that may contribute to more SCEs than the smaller Y chromosome in males. However, hormonal differences may also con-

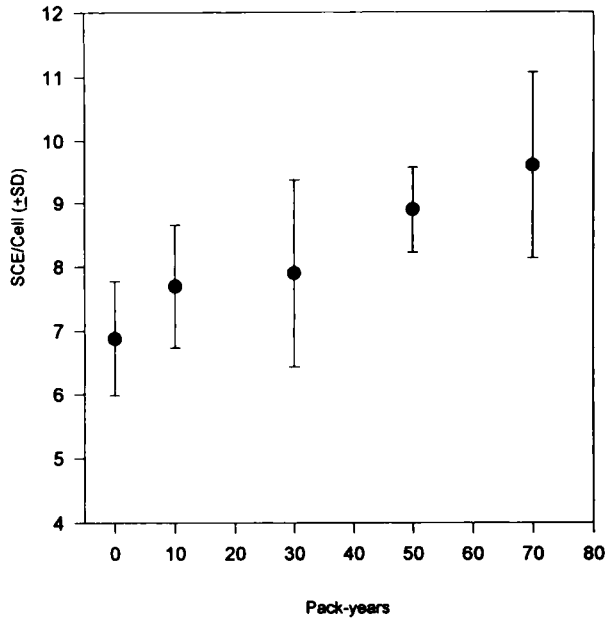

Fig. 1. SCE frequency in current and never smokers by pack-years smoked.

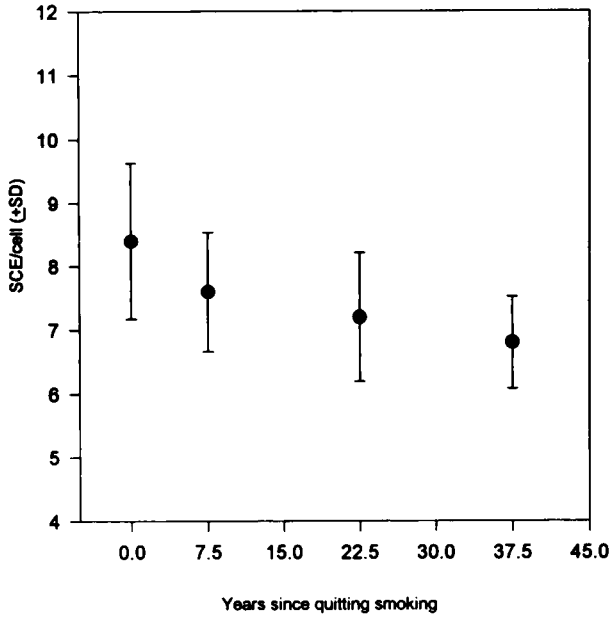

Fig. 2. SCE frequency in current and former smokers by the number of years since quitting smoking.

tribute to this increase of SCE frequency. In this study, SCE frequency increased with age until age 65 years. Previous studies reported that SCE frequency increased with age (48, 53, 54). The mean age of study subjects in previous studies was much lower than that of the current study. Lymphocyte number and subsets are known to differ in the young and the elderly (55). Additionally, the proliferation of lymphocytes in the elderly is also different from young individuals (56). Thus, the alteration of lymphocyte subsets and their proliferation in the elderly may affect SCE frequency.

Table 4 Multiple regression model for HFCs

	Regressiosn coefficient	SE	*P*
Intercept	16.0	1.1	<0.01
Age1[a]	0.49	0.54	0.4
Age2[b]	0.11	0.66	0.9
Gender (male)	−1.5	0.5	<0.01
Pack-yrs	0.034	0.008	<0.01
Yrs-quit	−0.036	0.017	0.03
GST-μ-null genotype	0.77	0.49	0.11
Vitamin A (1000 IU/day)	−0.035	0.025	0.16

[a] Reference group, age ≥ 65 years; age1 = 65 years.
[b] Reference group, age ≥ 65 years; age 2 ≤ 50 years.

The intake of vitamin A and selenium were inversely associated with SCE frequency in this study. Vitamin A has components of retinol and carotenoids. Carotenoids and selenium as antioxidants may quench free radicals in cigarette smoke. Although many epidemiological studies reported an association of antioxidants with lung cancer, no data have shown that SCE frequency is associated with either the intake or the plasma level of vitamin A and selenium (26, 34). Alcohol and coffee consumption were not associated with SCE frequency in this study. Several studies have reported small effects of alcohol (57, 58) and caffeine (59), but this has not been confirmed by others (53, 60).

In our study, SCE in GST-μ-null subjects was significantly higher than in those who had one or more alleles (GST-μ positive) after controlling for confounders. The slopes of smoking "dose" and SCE between GST-μ-positive and GST-μ-null genotypes were not statistically different. van Poppel (26) reported that previously heavy smokers who had the GST-μ-null genotype had higher SCEs. Thus, additional studies are needed to determine the role of smoking in the association of SCE frequency and GST-μ polymorphism.

The polymorphisms in CYP1A1 and CYP2D6 were not associated with SCE frequency in this study. This is not surprising, however, because the power to detect any effect of these genes on SCE is relatively small. There is <50% power to detect a difference of 1 SCE/cell among those with a variant CYP1A1 or CYP2D6 polymorphism at 5% significance level with our current sample size. Furthermore, no synergistic effect of GST-μ with CYP1A1 or CYP2D6 could be demonstrated in the current study.

HFC has been shown in some studies to be a more sensitive indicator than mean SCE in assessing chronic exposure to mutagens or carcinogens (61, 62). The current study showed that mean SCE was as good as HFC in assessing the association of smoking with changes in SCE but better in assessing the small effect of vitamin A and metabolic traits on SCE frequency.

In conclusion, we found that the GST-μ-null genotype was associated with higher SCEs, regardless of smoking status, and a higher consumption of vitamin A and selenium was inversely associated with SCE frequency.

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